Cah. Biol. Mar. (1997) 38 : 169-174

Morphometrics and energetic value of Adriatic ascidians.

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Abstract: Knowledge of the energy stored in communities is important in ecology. However, the determination of energetic content is laborious and time consuming. In order to facilitate the laboratory work done to obtain these data, some relationships between length, volume, surface, wet and dry weights and ash free dry weight were calculated regarding 18 species of ascidians. In addition, as the few energetic values known for ascidians are low and outside the energetic range common to the majority of other animal taxa, the energetic content of six ascidian species, was determined by analysing their biochemical composition. Our analyses showed that ascidian energetic values are included in the normal range of other animals but are located in its upper limit, and even higher in three species.

Résumé : *Morphométrie et contenu énergétique de quelques ascidies de la mer Adriatique.*
La connaissance de l'énergie accumulée dans les communautés biologiques est importante en écologie, mais l'analyse du contenu énergétique est longue et laborieuse. Afin de faciliter le travail de laboratoire nécessaire pour obtenir ces données, les relations entre longueur, volume, surface, poids humide, poids sec et poids sec sans cendres, ont été déterminées sur 18 espèces d'ascidies. En outre, comme les quelques données disponibles sur les contenus énergétiques des ascidies sont inférieures à celles indiquées pour la majorité des autres taxons animaux, les contenus énergétiques de six espèces d'ascidies ont été déterminés par l'analyse de leur composition biochimique. Les résultats indiquent que les valeurs énergétiques des ascidies sont situées dans la gamme normale des valeurs des autres taxons mais elles se placent dans la limite supérieure, ou même au-dessus de celle-ci pour trois espèces.

Keywords : Ascidians, morphometric measurements, biochemical composition, energetics.

Introduction

One of the main goals in the study of marine benthic communities is to express the data in biomass units. This is essential for a good description of the community structure and for the evaluation of the energetic content (joule.m^{-2}) of the benthic system. The biomass should be correctly expressed as ash free dry weight (AFDW) but amassing these data is tedious and time consuming. For this reason, studies on ecological energetics are thus usually performed by estimating only wet weight or dry biomass and then converting these data into energetic units by means of equivalent values. The aim of this work is to establish relationships between some measures that are easy and rapid to obtain such as wet weight, volume, length and surface and the dry weight (DW) or the AFDW of the whole organism. The study was performed on several ascidian species, among the most abundant, present in hard bottom communities of the northern Adriatic Sea (Brunetti, 1994; Gabriele *et al.*, unpublished). In addition, we analysed the biochemical composition of some of these animals to obtain

Reçu le 29 juillet 1997 ; accepté après révision le 25 septembre 1997.
Received 29 July 1997 ; accepted in revised form 25 September 1997.

a precise conversion factor from AFDW to energetic content values (J). This was done because the few data available from the scientific literature indicate ascidians as the only taxon with an energetic value outside the range common to all other marine taxa analysed (Brey *et al.,* 1988; Wacasey & Atkinson, 1987).

Materials and methods

Samples were collected in different seasons from July 1994 to May 1996 by means of SCUBA diving. When possible, a high number of animals of different sizes, extending over a wide dimensional range, was sampled.

Morphometric measurements: The animals were anaesthetized by addition of some crystals of menthol and novocaine to the sea water, in order to avoid the contraction of the body, which could affect the measurements and make taxonomic analysis difficult. Then they were carried alive to the laboratory in the sea water mixture at a controlled temperature. Wet weight was measured after removing the water contained in the body cavities and mopping the body surface with blotting paper. The DW was obtained by heating the organisms in an oven at about 100 °C until they reached a constant weight. The ash content was determined by burning off the organic matter in a muffle furnace for about five hours at 550 °C as recommended by Crisp (1984). The volume of *Polycitor adriaticus* was determined by displacement of water (the small volume of branchial cavities in this colonial species do not affect the value of the measurement), the length of anaesthetized individuals was measured to the nearest mm. The relationships were calculated by regressing the $\log_{10}$ of the measurements, according to the formula: $\log_{10} w = a + b \log_{10} m$, where w is the AFDW and m is the value of the considered measurement.

Biochemical and energetic composition: sampled animals were immediately frozen by means of dry ice and stored in the laboratory at a temperature of -80 °C. Then they were lyophilized and powdered using a mortar. A preliminary set of experiments was performed to obtain the calibration curves for carbohydrates, proteins and lipids using increasing amounts of glucose, albumin and stearic acid respectively. Carbohydrate determination was done following the technique suggested by Dubois *et al.* (1956) while lipids were extracted and measured following those of Mann & Gallagher (1985) and Pande *et al.* (1963) respectively. NaOH soluble proteins were determined with the method of Lowry e*t al.* (1951) and insoluble proteins were calculated by subtraction (Lawrence, 1973; McClintock *et al.*, 1991). Energetic value (J.mg^{-1}) was obtained indirectly by multiplying the DW or the AFDW by the percentage of each organic class and its energetic equivalent (Brody, 1945). Biochemical composition values, organic content and energetic values represent the mean of all available individuals. For each sample, determinations were made in triplicate.

Results

Morphometric measurements

The regression statistics for the measurements performed are reported in Table 1. In all cases a highly significant statistical correlation was found (significance level ranging from $P<0.01$ to $P<0.001$). Moreover in Fig. 1 the regression lines between wet weight and ash free dry weight for the species used for biochemical analyses are shown.

Biochemical and energetic composition

The results of the biochemical analyses on lyophilized samples are presented in Table 2. Levels of lipids were generally low in all species (2.35 ÷ 3.98 % DW) while carbohydrate content varied greatly in the different species: the lowest levels were found in *Botrylloides violaceus* (3.27 % DW) and *Botryllus schlosseri* (3.53 % DW) while the highest amount was present in *Polycitor adriaticus* (10.7 % DW). In all the species analysed the prevalence of the proteinaceous component is evident, particularly that belonging to the insoluble fraction. Collectively, proteins (soluble and insoluble) made up the most prevalent organic component in all species, with values ranging from 26.02 % DW *(Aplidium conicum)* to 41.94 % DW *(Polycitor adriaticus)*.

The organic content as a percentage of DW, and energy values expressed as J.mg^{-1} DW and J.mg^{-1} AFDW, are shown in Table 3. Energetic values such as J.mg^{-1} AFDW vary from a minimum of 23.3 (*Polycitor adriaticus*) to a maximum of 24.16 (*Botryllus schlosseri* and *Aplidium elegans*). With regard to the energetic values expressed as J.mg^{-1} DW, the highest value is that of *Polycitor adriaticus*, as a consequence of its low ash content, while *Aplidium conicum* which has the highest ash content shows the lowest value (Table 2). The regression of energetic values per DW unit on percentages of organic content of the six ascidians analysed is shown in Fig. 2. The regression equation, $y = 0.8954 + 0.2193x$, may be used to predict energetic values of the DW units from those of the organic content.

Discussion

The calculation of relationships between readily obtainable body measurements and AFDW is very useful in simplifying and shortening laboratory work. In addition, the knowledge of conversion factors from DW or AFDW to J allows energetic values to be obtained by measuring the wet weight (or another readily obtainable measure). It is evident from Table 3 that there is more variability in values of J.mg^{-1} DW than in those of J.mg^{-1} AFDW; thus, if a conversion factor for ascidians is desired, it should be calculated only on AFDW values.

Table 1. Regression statistics. All the measurements have been $\log_{10}$ transformed. ww = wet weight (g); dw = dry weight (g); afdw = ash free dry weight (g); surf. = surface (cm^2); p. a. = product of axes (cm).

Tableau 1. Statistiques de la régression. Toutes les mesures ont été transformées en $\log_{10}$. ww = poids humide (g) ; dw = poids sec (g) ; afdw = poids sec sans cendres (g) ; surf = surfaces (cm^2) ; p. a. = produit des axes (cm).

Species	log x	log y	a	b	R	n	P	s. e.	t
Distaplia sp.	ww	afdw	-1.3274	0.9795	0.9963	6	< 0.001	0.042	23.25
Della Valle	dw	afdw	-0.3719	1.1486	0.9916	6	< 0.001	0.075	15.37
Lissoclinum perforatum	surf	afdw	-2.2953	1.1712	0.9935	9	< 0.001	0.050	23.04
(Giard)	ww	afdw	-1.2483	0.9445	0.9930	9	< 0.001	0.042	22.32
	dw	afdw	-0.4555	0.9673	0.9986	9	< 0.001	0.019	49.81
Polycitor adriaticus	vol.	afdw	-1.6226	1.0579	0.9940	11	< 0.001	0.039	27.19
(von Drasche)	ww	afdw	-1.4868	0.9922	0.9952	18	< 0.001	0.024	40.72
	dw	afdw	-0.3496	0.9623	0.9899	11	< 0.001	0.035	27.88
Cystodites dellechiajei	surf	afdw	-2.1871	1.2572	0.9977	6	< 0.001	0.043	29.41
(Della Valle)	ww	afdw	-1.5810	0.9712	0.9900	15	< 0.001	0.038	25.30
	dw	afdw	-0.4079	0.9573	0.9966	15	< 0.001	0.022	43.52
Eudistoma mucosum	ww	afdw	-1.5699	0.8702	0.9924	7	< 0.001	0.048	18.08
(von Drasche)	dw	afdw	-0.4470	0.9207	0.9868	7	< 0.001	0.067	13.65
Aplidium conicum	ww	afdw	-1.6320	1.0526	0.9531	15	< 0.001	0.093	11.35
(Olivi)	dw	afdw	-0.3439	0.9524	0.9492	15	< 0.001	0.088	10.88
Aplidium elegans	ww	afdw	-1.5012	0.9707	0.9981	16	< 0.001	0.016	60.28
(Giard)	dw	afdw	-0.3674	1.0180	0.9975	16	< 0.001	0.019	52.49
Ciona intestinalis	ww	afdw	-2.0100	1.0266	0.9963	7	< 0.001	0.049	26.02
(Linnaeus)	dw	afdw	-0.4065	1.0721	0.9960	7	< 0.001	0.043	24.85
Phallusia fumigata	ww	afdw	-1.5671	1.1142	0.9669	6	<0.01	0.147	7.58
(Grübe)	dw	afdw	-0.3241	1.0496	0.9722	6	< 0.01	0.126	8.31
Phallusia mammillata	ww	afdw	-1.9751	1.1542	0.9937	6	< 0.001	0.065	17.74
(Cuvier)	dw	afdw	-0.6021	1.2560	0.9931	6	< 0.001	0.074	16.91
Ascidiella aspersa	ww	afdw	-1.4814	0.9209	0.9852	9	< 0.001	0.061	15.21
Müller	dw	afdw	-0.4615	0.8525	0.9871	9	< 0.001	0.052	16.34
Styela plicata	p. a.	afdw	-1.8520	1.1644	0.9911	17	< 0.001	0.040	28.99
(Lesueur)	ww	afdw	-1.4094	0.9120	0.9969	17	< 0.001	0.019	48.93
	dw	afdw	-0.2712	0.9405	0.9972	17	< 0.001	0.018	52.11
Pyura dura	ww	afdw	-0.9488	0.9099	0.9838	10	< 0.001	0.059	15.54
(Heller)	dw	afdw	-0.4243	0.7764	0.9942	10	< 0.001	0.030	26.14
Microcosmus vulgaris	ww	afdw	-1.0402	0.9530	0.9930	25	< 0.001	0.024	40.32
Heller	dw	afdw	-0.1870	0.8753	0.9755	25	< 0.001	0.041	21.24
Microcosmus polymorphus	ww	afdw	-1.2610	1.0480	0.9980	18	< 0.001	0.017	62.40
Heller	dw	afdw	-0.4465	1.0299	0.9911	18	< 0.001	0.035	29.73
Molgula socialis	ww	afdw	-1.4315	1.0092	0.9806	18	< 0.001	0.050	20.00
Alder	dw	afdw	-0.4191	1.0287	0.9939	18	< 0.001	0.029	35.99
Botrylloides violaceus	surf	afdw	-2.9153	1.6321	0.9603	6	< 0.01	0.237	6.88
Oka	ww	afdw	-1.3302	0.9202	0.9684	6	< 0.01	0.118	7.77
	dw	afdw	-0.2553	0.9513	0.9910	6	< 0.001	0.064	14.82
Botryllus schlosseri	surf	afdw	-2.5963	1.2361	0.9907	6	< 0.001	0.085	14.57
(Pallas)	ww	afdw	-1.3986	0.9371	0.9919	7	< 0.001	0.054	17.43
	dw	afdw	-0.4615	0.8165	0.9764	7	< 0.001	0.081	10.11

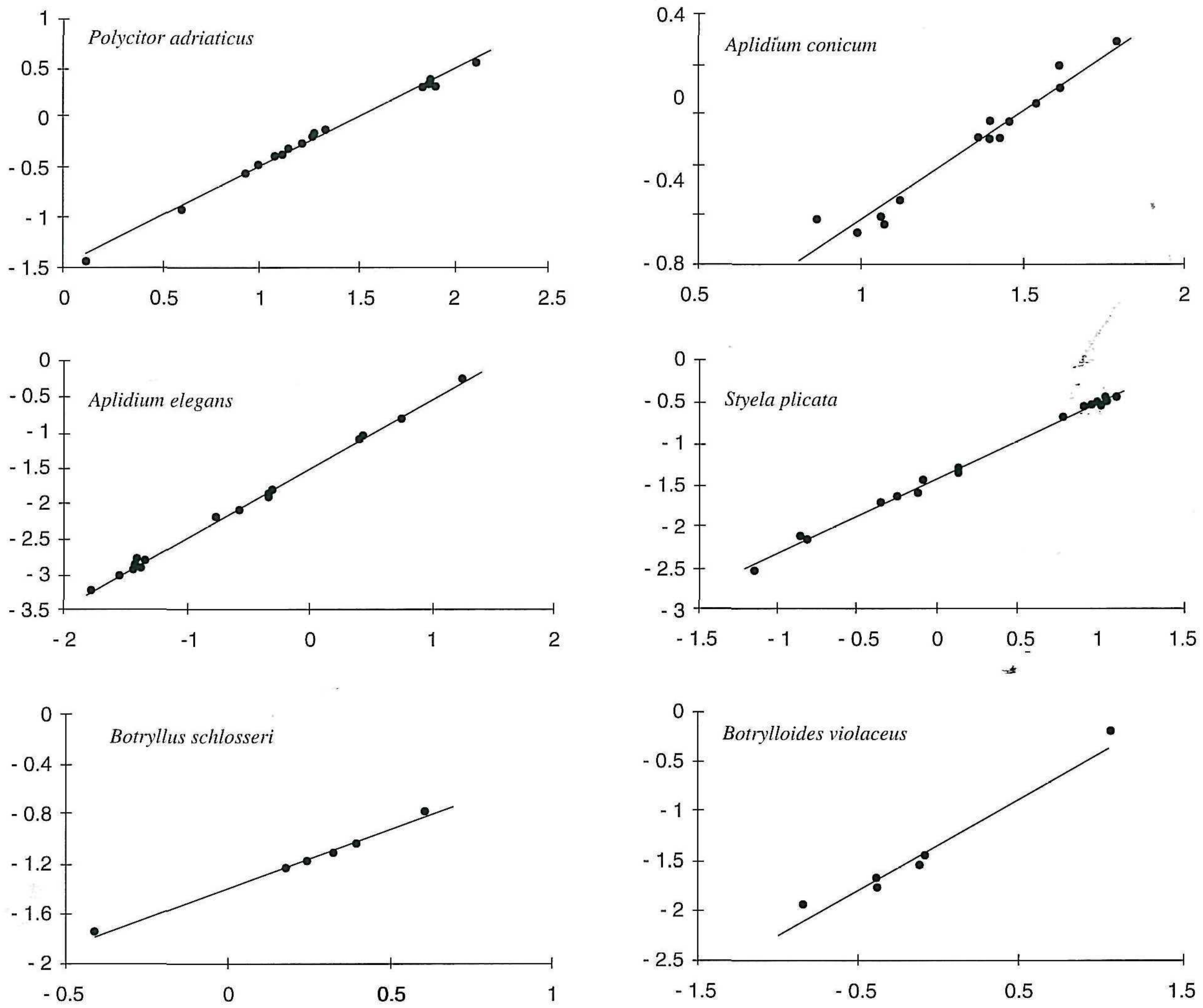

Figure 1. Log_{10} ash free dry weight (ordinate) *vs* log_{10} wet weight (abcissa) in the six species used for biochemical analyses. Weights in grams. For regression statistics see Table I.

Figure 1. Log_{10} du poids sec sans cendres (ordonnée) *vs* log_{10} du poids frais (abscisse) pour les six espèces analysées biochimiquement. Poids en grammes. Pour les statistiques de la régression voir le Tableau 1.

The energetic content of organisms is due to the amounts of its organic components: carbohydrates, proteins and lipids. The ratio of these compounds, if inorganic components are ignored, determines the energy content per unit of body mass. Biochemical analyses on our ascidian species show that most of the organic material is in the form of proteins, particularly the so-called "insoluble proteins" (that is proteins not soluble in NaOH). As this category of proteins was calculated by subtraction, it is possible, as already reported by McClintock *et al.* (1991), that some fraction of the material considered to belong to this category is a protein-polysaccharide complex, as observed in other ascidian species by Smith & Denhel (1970, 1971). This eventuality might lead to an overestimation of the energetic content, as a consequence of the higher caloric value of proteins with respect to carbohydrates. Actually, the energetic values obtained by us range from 23.33 (*Polycitor adriaticus*) to 24.16 (*Botryllus schlosseri* and *Aplidium elegans*) $J.mg^{-1}$ AFDW. These values are higher than those reported by literature for other ascidians (Wacasey & Atkinson, 1987; Brey *et al.*, 1988). However, it is necessary to point out that in the papers quoted above the energetic

Table 2. Biochemical composition (% dry weight) of the ascidians studied. Averages ± 95 % confidence intervals are given.
Tableau 2. Composition biochimique (% poids sec) des ascidies étudiées. Les moyennes ± l'intervalle de confiance (95 %) sont indiquées.

Species	n	Carbohydrates	Lipids	Proteins	Insoluble proteins	Ash
Polycitor adriaticus	6	10.7 ± 0.72	3.24 ± 1.10	16.42 ± 1.35	25.52 ± 2.60	44.12 ± 2.42
Aplidium conicum	6	5.18 ± 1.20	3.16 ± 1.26	10.87 ± 1.27	15.15 ± 1.60	65.64 ± 5.68
Aplidium elegans	3	6.05 ± 1.65	3.98 ± 0.27	18.51 ± 1.64	18.8 ± 2.64	52.66 ± 1.80
Styela plicata	3	9.72 ± 0.05	3.19 ± 0.79	18.25 ± 1.84	22.62 ± 11.24	46.22 ± 8.56
Botryllus schlosseri	3	3.53 ± 0.65	2.87 ± 0.54	15.21 ± 0.62	23.02 ± 6.82	55.37 ± 8.07
Botrylloides violaceus	3	3.27 ± 0.53	2.35 ± 0.44	15.45 ± 1.02	26.05 ± 1.34	52.88 ± 0.58

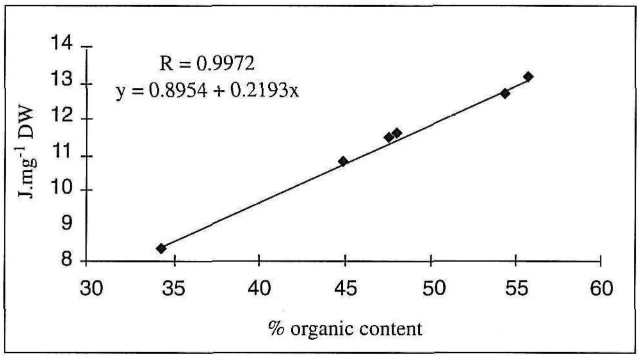

Figure 2. DW energetic value *vs* percentage organic content of the six ascidians analysed. Statistics: s.e. = 0.008; t = 26.54; P < 0.001.

Figure 2. Valeur énergétique du poids sec *vs* le pourcentage du contenu en matière organique pour les six ascidies analysées. Statistiques : s.e. = 0.008 ; t = 26.54 ; P < 0.001.

Table 3. Organic content and energetic values in the ascidians studied. Averages ± 95 % confidence intervals are given. The collection season is indicated: wi = winter; su = summer; sp = spring; wi + su = winter + summer data pooled.

Tableau 3. Contenu en matière organique et valeurs énergétiques des ascidies étudiées. Les moyennes ± l'intervalle de confiance (95 %) sont indiquées : wi = hiver ; su = été ; sp = printemps ; wi + su = données d'hiver et d'été rassemblées.

Species	season	n	Organic % DW	$J.mg^{-1}$ DW	$J.mg^{-1}$ AFDW
Polycitor adriaticus	wi + su	6	55.88 ± 2.42	13.04 ± 0.49	23.33 ± 0.27
Aplidium conicum	wi + su	6	34.35 ± 5.67	8.29 ± 1.46	24.10 ± 0.30
Aplidium elegans	wi	3	47.33 ± 1.83	11.43 ± 0.47	24.16 ± 0.30
Styela plicata	sp	3	53.79 ± 8.56	12.60 ± 1.90	23.42 ± 0.18
Botryllus schlosseri	sp	3	44.64 ± 8.08	10.78 ± 1.97	24.16 ± 0.05
Botrylloides violaceus	sp	3	47.12 ± 0.58	11.31 ± 0.19	23.99 ± 0.22

Further data on energetic value of other ascidian species may be found in Wacasey & Atkinson, 1987; Brey *et al.*, 1988; McClintock *et al.*, 1991.

content was calculated almost exclusively in solitary species, while our data, with the exception of *Styela plicata*, were obtained from colonial species. Studying the geographical variability of energetic values Griffiths (1977) postulated that, reacting to decreasing environmental stability encountered in increasing latitudes, organisms should tend to store higher amounts of energy and thus to have higher caloric values. Other studies (Clarke, 1983), however, indicate no increase in energy storage with increasing latitudes. Considering that the northern Adriatic shows a strong thermal variation in changing seasons, our data on the two species (*Polycitor adriaticus* and *Aplidium conicum*) typical of this marine area (Gabriele *et al.*, unpublished) would seem to support Griffiths' conclusions. In fact in both these species the lipidic content increases significantly (P < 0.05) in winter and consequently increases the caloric content (Fig. 3).

Slobodkin & Richman (1961) hypothesized that energetic values for different animal taxa seem to indicate a natural pattern that is symmetrical about a mean. That would suggest a selection against caloric values which deviate in either direction from this mean value. Further literature data confirm this idea and strongly indicate that this mean value is in the range of 22.60 to 23.85 $J.mg^{-1}$ AFDW (Wacasey & Atkinson, 1987). Our values, in four cases out of six (*Aplidium conicum, A. elegans, Botryllus schlosseri* and *Botrylloides violaceus*) are higher than the upper limit of this range while the other three species show values within the range but near to its upper value (Table 3). These results are in disagreement with those of Wacasey & Atkinson (1987), who report Ascidiacea as the only taxon with a lower caloric content that is outside the reported range. That might be ascribed to the particular composition of the ascidians test, which is the only animal tissue containing carbohydrates in cellulose form. In fact, as shown by Smith & Denhel (1971), the carbohydrate fraction of the test varies from 37 to 67 % in different species and this might positively affect our energetic data. However, even if we consider that about 50 % of our insoluble proteins are in reality represented by polysaccharides, mean values of energetic content will remain within the range quoted above or are considerably higher than those reported by Brey *et al.* (1988) and Wacasey & Atkinson (1987).

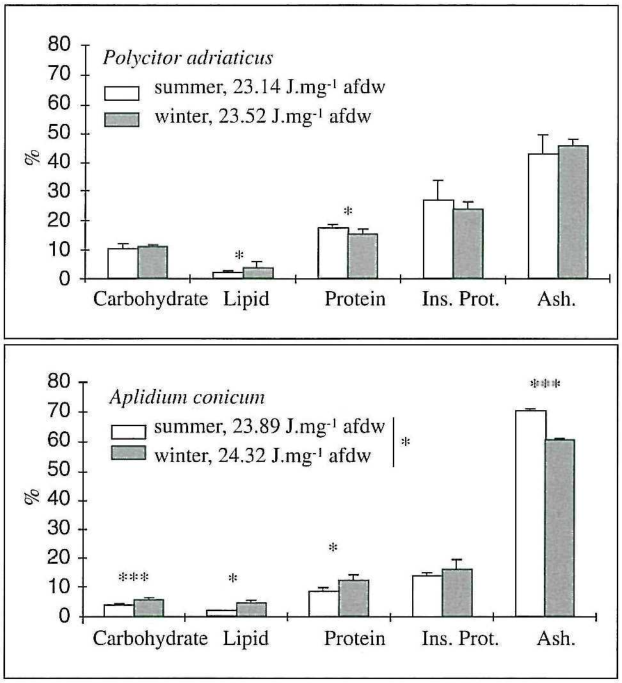

Figure 3. Biochemical composition (Averages and 95% confidence intervals; n = 3) of two ascidians, *Polycitor adriaticus* and *Aplidium conicum*, in different seasons. Asterisks indicate significant statistical difference (ANOVA): * = P<0.05; *** = P<0.001.

Figure 3. Composition biochimique (moyenne et intervalles de confiance à 95 % ; n = 3) des ascidies *Polycitor adriaticus* et *Aplidium conicum* à différentes saisons. Les astérisques indiquent la signification statistique (ANOVA) : * = P<0.05 ; *** = P<0.001.

Acknowledgements

This study was supported by grants from the Italian *Ministero per l'Università e la Ricerca Scientifica e Tecnologica* (M.U.R.S.T). The authors wish to thank the 7th Legion of the "*Guardia di Finanza*" of Venice for facilities, in particular Col. F. Manucci, Cap. D. Capano and M.llo S. Longone. Finally, we wish to thank the two anonymous reviewers for their constructive suggestions.

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